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Supplemental Information

Probing the Basis of α-Synuclein Aggregation by Comparing Simulations to Single-Molecule Experiments

Cassandra D.M. Churchill, Mark A. Healey, Jordane Preto, Jack A. Tuszynski, and Michael T. Woodside

Supplementary Methods:

The terms of the interaction potential in Eq. 2, $E = E_{\mathrm{loc}} + E_{\mathrm{ev}} + E_{\mathrm{hb}} + E_{\mathrm{hp}}$, are described in detail in Refs. 70 and 71. The equations describing these terms are given below.

The first term, describing electrostatic effects, is given by

$$E_{\mathrm{loc}} = \kappa_{\mathrm{loc}} \sum_{I} \left(\sum_{ij} \frac{q_i q_j}{r_{ij}^{(I)}} \right), \tag{S1}$$

where $q_{i,j}$ are the partial charges of the backbone NH and C′O groups in a given amino acid I, r_{ij} is the distance between the partial charges, $\kappa_{\mathrm{loc}} = 100$ is a constant related to the dielectric constant, and the external sum is over all amino acids.

The second term, describing excluded-volume effects, is given by

$$E_{\mathrm{ev}} = \kappa_{\mathrm{ev}} \sum_{i<j} \left[\frac{\lambda_{ij}\left(\sigma_i + \sigma_j\right)}{r_{ij}} \right]^{12}, \tag{S2}$$

where the summation is over all pairs of atoms (i, j), r_{ij} is the distance between atoms, σ_i are constants differing for each atom, λ_{ij} is 0.75 for all pairs except those with 3 covalent bonds where it is 1, and $\kappa_{\mathrm{loc}} = 0.1$ is a constant.

The third term, describing hydrogen bond energies, is given by

$$E_{\mathrm{hb}} = \varepsilon_{\mathrm{hb}}^{(1)} \sum_{\mathrm{bb-bb}} u\left(r_{ij}\right) v\left(\alpha_{ij}, \beta_{ij}\right) + \varepsilon_{\mathrm{hb}}^{(2)} \sum_{\mathrm{sc-bb}} u\left(r_{ij}\right) v\left(\alpha_{ij}, \beta_{ij}\right), \text{ with}$$

$$u(r) = 5\left(\frac{\sigma_{\mathrm{hb}}}{r}\right)^{12} - 6\left(\frac{\sigma_{\mathrm{hb}}}{r}\right)^{10} \text{ and } v(r) = \begin{cases} (\cos\alpha \cos\beta)^{1/2}, \alpha, \beta > 90^{\circ} \\ 0, \alpha, \beta \le 90^{\circ} \end{cases}. \tag{S3}$$

Here, only hydrogen bonds between NH and CO groups and included, r_{ij} is the O–H distance, α_{ij} is the NHO bond angle, β_{ij} is the HOC bond angle, $\varepsilon_{\mathrm{hb}}$ and σ_{hb} are constants, the first sum is taken over backbone-backbone interactions, and the second sum is taken over sidechain-backbone interactions.

The fourth term, describing an effective hydrophobic interaction between non-polar sidechains, is given by

$$E_{\mathrm{hp}} = \varepsilon_{\mathrm{hp}} \sum_{I<J} M_{IJ} C_{IJ}, \tag{S4}$$

where the sum is taken over all pairs of non-polar sidechains, $\varepsilon_{\mathrm{hp}}$ is a constant, M_{IJ} is a matrix of hydrophobicity constants, and C_{IJ} is a measure of the extent of contact between sidechains calculated as described in Refs. 70 and 71.

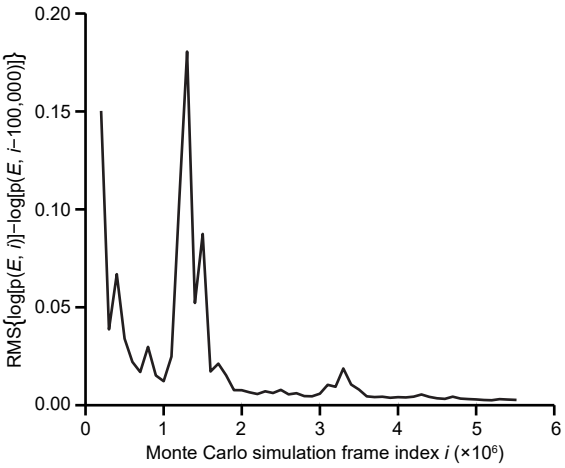

Figure S1: Convergence of structural ensemble in Monte Carlo simulations. Convergence of the ensemble was tested by extracting the energy distribution $p(E)$ after every 100,000 steps in the simulation and then calculating the rms difference between the logarithm of successive energy distributions as the simulation progressed. This difference became close to 0 above 5 million steps, indicating convergence.

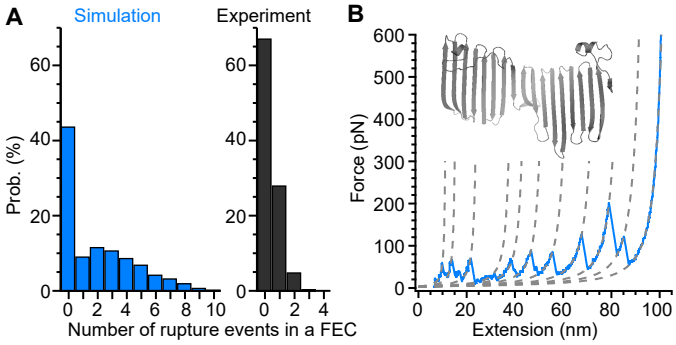

Figure S2: Rupture events in force-extension curves. (A) A larger number of rupture events is seen in simulated FECs (blue) compared to experimental FECs (black). (B) A simulated FEC showing 10 discrete rupture events during unfolding of an ordered structure containing 65% β-sheet content. Each branch of the FEC was fit to a WLC (dashed lines).

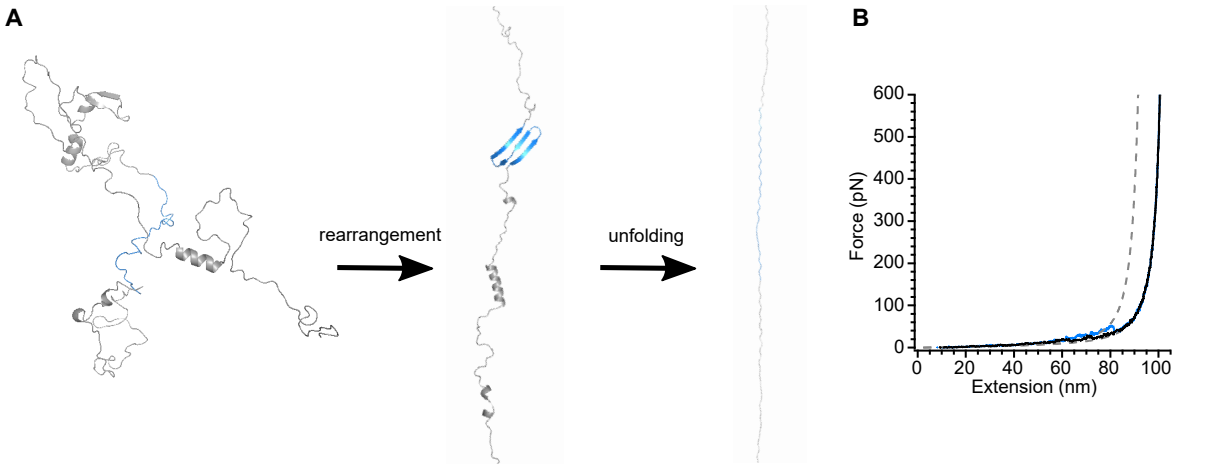

Figure S3: Structural rearrangement during simulated pulling. (A) Rarely, simulated pulling of an α-synuclein dimer with little secondary structure rearranged during pulling to form a force-resistant metastable β-sheet (blue). (B) FECs resulting from pulling such structures typically show no discrete rupture events (blue), but occasionally a replicate features a low-force rupture (blue) corresponding to the unfolding of the newly-formed β-sheet (as in A). Dashed line: WLC fit.

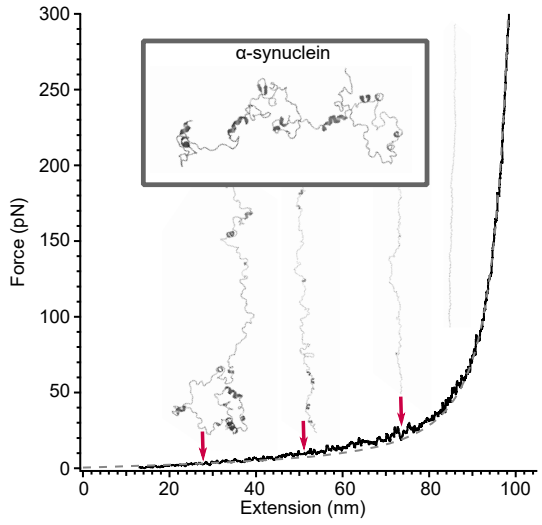

Figure S4: Non-cooperative unfolding of helical conformers. A simulated FEC for an α-synuclein dimer with 24% α-helical character shows unfolding that occurs via continuous, non-cooperative transitions, producing a FEC without discrete rupture event. The structures of the dimer are illustrated at various point along the unfolding trajectory.

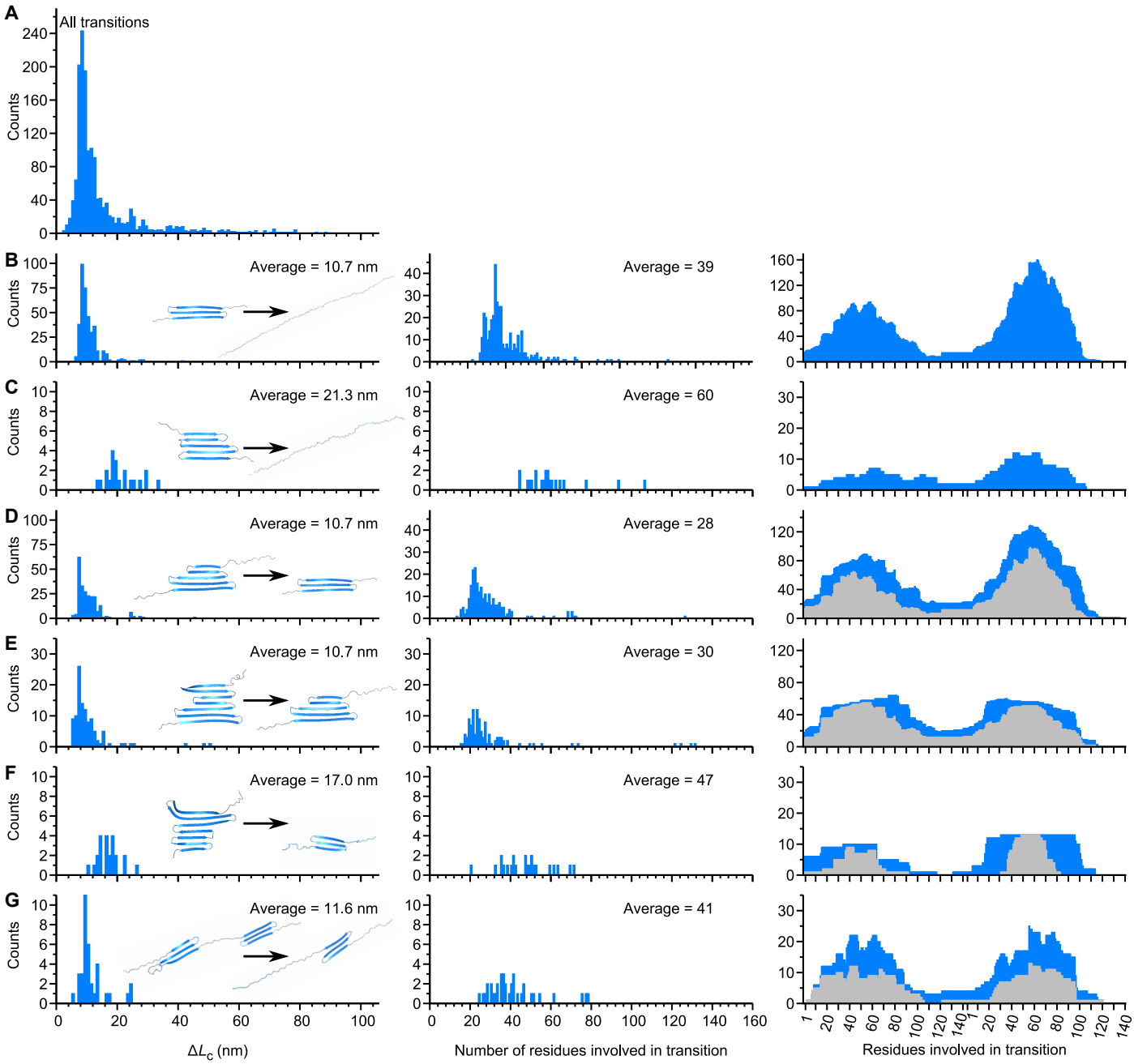

Figure S5: Analysis of select structural transitions. Left column: L_c distributions from (A) all simulations and (B–G) select structural transitions for unfolding anti-parallel β-strands (structures illustrated in insets). Center column: The number of residues that lost secondary structure during the unfolding event. Right column: The residues with secondary structure before (blue) and after (grey) the structural transition.

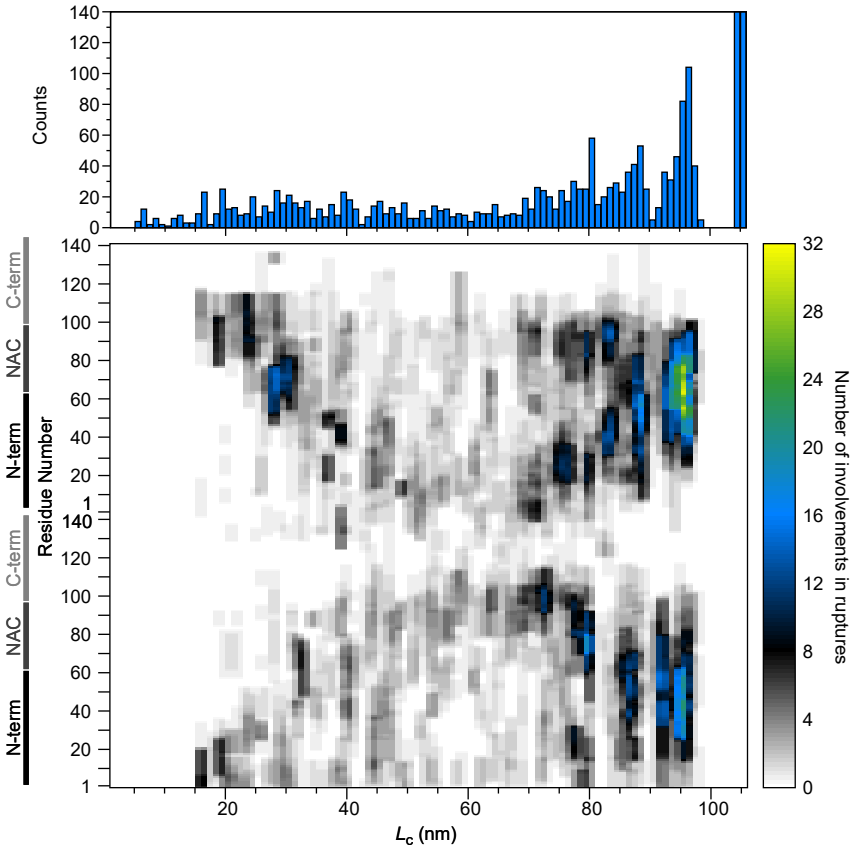

Figure S6: Map of residues involved in rupture events. A contour plot of the residues that lose secondary structure during rupture events at each L_c value shows that the N-terminal and NAC regions are more likely to form secondary structures generating rupture events whereas the C termini and the linker region are less likely to do so. Left: schematic of protein domains. Top: Histogram of L_c for all rupture events in FECs.

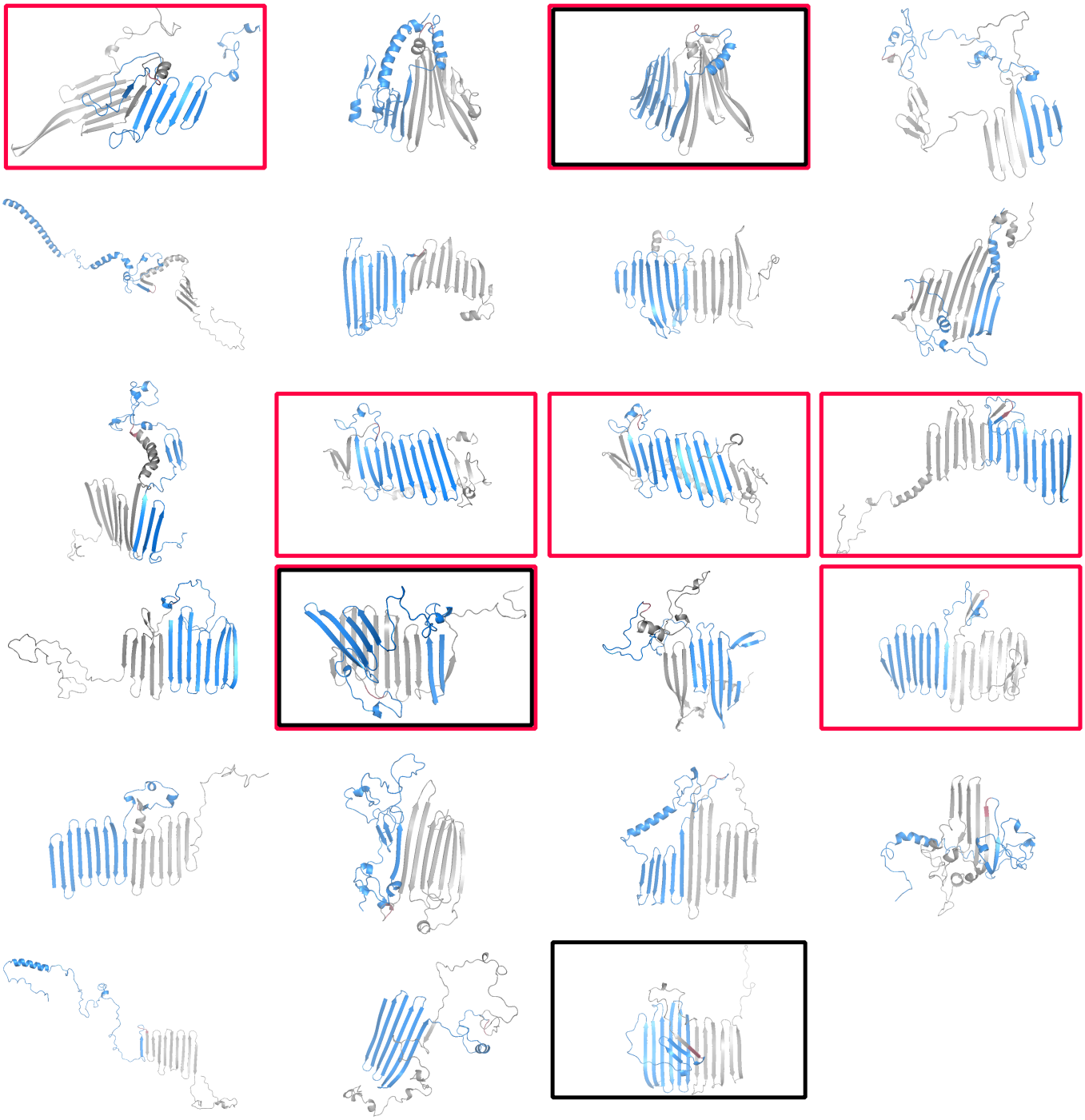

Figure S7: Structures containing interfaces between domain 1 (blue) and domain 2 (grey), with the linker region indicated in pink. The interfaces identified in our work primarily feature edge-to-edge interactions between sheets in different domains. Some structures contain two edge-to-edge interfaces (red box), while other structures have an interface formed face-on between sheets from each domain (black box).

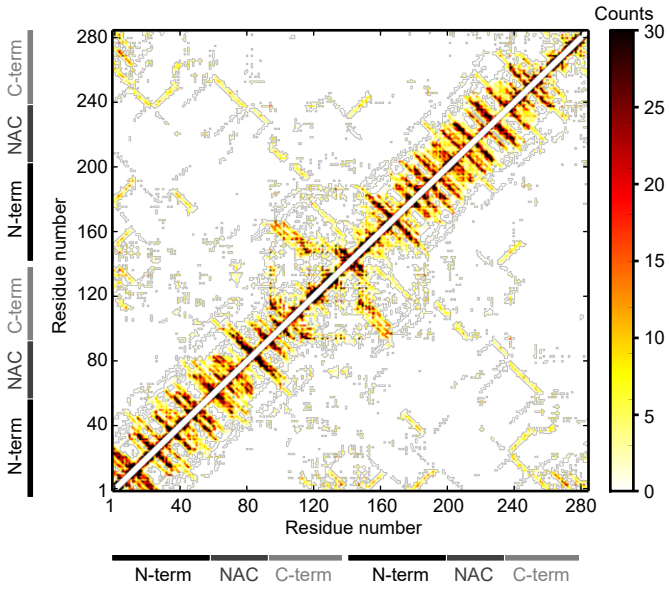

Figure S8: Full contact map for dimer structures. Contact map built from all 266 pulling trajectories showing discrete ruptures at the interface, showing all contacts (interfacial and non-interfacial).

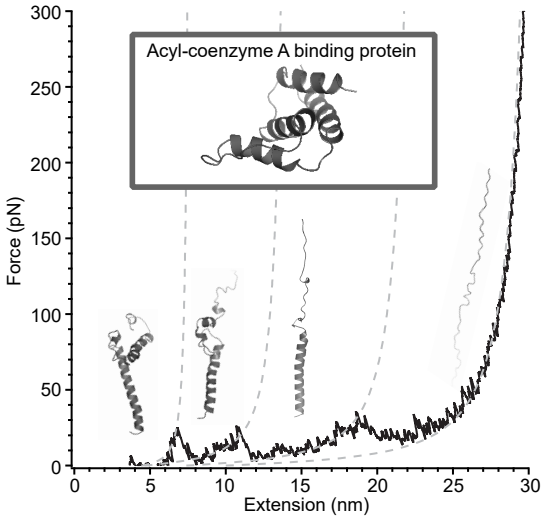

Figure S9: Simulated pulling of an α-helical protein. Simulated FECs of the unfolding of acyl-coenzyme A binding protein (ABP) obtained using the same simulation conditions as for α-synuclein dimers show discrete rupture events, in contrast to the non-cooperative unfolding seen in helical conformers of α-synuclein dimers. Unfolding transitions can be fit by WLCs (dashed lines), and rhe structures corresponding to each branch of the FEC are illustrated.